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Crystallography: Deep Understanding the Mechanism of β-form Nucleation in Cooling Crystallization of L-glutamic Acid

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Abstract

The mechanism of heterogeneous β-form nucleation was investigated during the phase transformation of L-glutamic acid in the stirring cooling crystallization. In the present study, a new mechanism of heterogeneous β-form nucleation was explored, where the β-form nuclei was favorably crystallized on the (001) and (011) surfaces rather than the (111) surface of α-form crystal during the phase transformation. This result was confirmed via the molecular simulation, in which the functional groups of molecule on various surfaces of α-form crystal were different, so the degree of lattice matching (E) between the α-form substrate surfaces and β-form molecule aggregate was distinguished and ordered as (001) > (011) > (111), meaning that the nucleation of heterogeneous β-form was more facilitated on the (001) and (011) surfaces compared to that on the (111) surface of α-form crystal.

Keywords. Crystallization, crystallography, polymorphism, nucleation, crystal growth.

1. INTRODUCTION

Crystallization is very important separation, purification and particle synthesis process used widely in pharmaceutical, chemical, and food industries, etc [1]. In the pharmaceutical industry, there are more than 90% of all pharmaceutical products containing the active pharmaceutical ingredients (API) in crystalline forms. As such, crystallization process is certainly required to make the phase transition of molecules from the solution to the solid state. Plus, the orderly structured crystalline facilitates impurity rejection from the solid product, so the purity of pharmaceutical product is significantly enhanced via the crystallization process, which is particularly important because the purity of this kind product is always required at least 98%. Moreover, during the crystallization, the solid product can adopt more than one crystal structure due to the different packing arrangement and conformation of molecules in the crystal lattice, and this phenomenon is known as the polymorphism, which is very common in organic compounds. Generally, there is more than 50% of API compounds having the polymorphism, and since the different polymorphic crystal has marked differences in the physico-chemical properties such as bio-availability, solubility, hardness, chemical stability, etc, control of polymorphism becomes a vital issue in any pharmaceutical crystallization process [2, 3].

Amino acid L-glutamic has a wide application in the pharmaceutical, chemical and food industries. L-glutamic acid has two kinds of polymorphic crystal including metastable α-form and stable β-form, where the mechanism of polymorphic nucleation is very complicated and elusive because it depends on so many crystallization conditions. For example, Tahri et al [4] reported that nuclei of α-form and β-form were both generated under the stirring condition, while the only β-form nuclei appeared under the stagnant condition, meaning that the fluid hydrodynamic condition in crystallizer is significant factor as it directly impacts on the mechanism of polymorphic nucleation. Lai et al [5] also reported that the α-form nuclei crystallized at a low temperature as 25^0C, while the only β-form nuclei performed at a high temperature as 45 ^{o}C in the continuous MSMPR crystallizer. According to Florence et al [6], in order to initiate the β-form nuclei at a low temperature as 25 ^{o}C, the seeding crystal is a valuable method when using the continuous Oscillatory baffles crystallizer.

In Vietnam, it is definitely confirmed that our material crystallization research is very unique, and of course it do not duplicate any other group's research. Plus, our current work has not been reported in any previous literature in the world. In present study, the nucleation of β-form L-glutamic acid was deeply investigated during phase

transformation via the experimental and molecular simulation in stirring cooling crystallization, so the mechanism of β-form nucleation would be more understood.

2. EXPERIMENTAL

The standard Stirred tank crystallizer was designed by Tuan et al [3]. The L-glutamic acid material (>98% purity) was bought from Sigma Aldrich company. The concentration of feed solution was 18.5(g/L), which was prepared by dissolving the material into the distilled water at 50 ^{o}C. Initially, the crystallizer was fully filled with the feed solution, and then operated as the batch mode in the cooling crystallization at 4.0 ^{o}C/min of cooling rate and 360rpm of agitation speed. Here, the temperature and agitation speed of crystallizer were controlled via the circulating coolant from the chiller and motor, respectively.

The suspensions were periodically taken from the crystallizers and quickly filtered by using a vacuum pump. The crystal samples were then dried in a desiccator and analyzed to define the shape, structure and crystal fraction of β-form. Here, the shape and structure of crystal product were monitored and confirmed by Video microscope and XRD patterns (M18XHF-SRA, Japan), respectively. Meanwhile, the molecular simulation was carried out via the crystallographic softwares including Encifer, Mercury, Diamon and Grace [7-9].

3. RESULTS AND DISCUSSION

3.1. Crystallography of α-form and β-form

The parameters and position of each molecule in unit cell of α-form and β-form were collected from the Cambridge Structural Database [9]. Although the crystal system of two polymorphs is orthorhombic with space group $P2_12_12_1$, the α-form crystal has a unit cell parameters as a = 7.068, b = 10.277, c = 8.755 Å, while the β-form crystal has a unit cell parameters as a = 5.159, b = 17.300, c = 6.948 Å. From these raw data, the Cif file was coded via the Encifer software, and then the molecules and unit cell were simulated via the Mercury software, as shown in Fig.1. Here, the crystallographic data showed distinction in terms of the unit cell parameters, packing arrangement and conformation of molecule of two polymorphs (Fig. 1). The difference of crystal structure of two polymorphs was also confirmed via the powder XRD pattern at reflective angles 2θ of 10^0, 15^0, 16^0, 18^0, 21^0, 23^0, 26.5^0, 27.5^0, as depicted in Fig.2. Moreover, the distinguished morphology of α-form and β-form crystals was clearly observed via the prism shape of α-form and needle shape of β-form, respectively, as shown in Fig. 2.

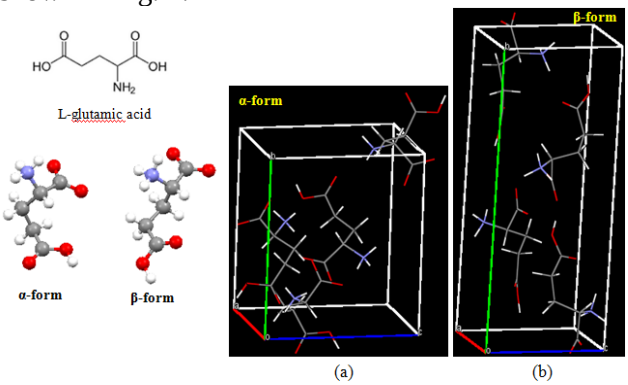

Figure 1: Conformation, packing and unit cell parameters of α-form and β-form crystal

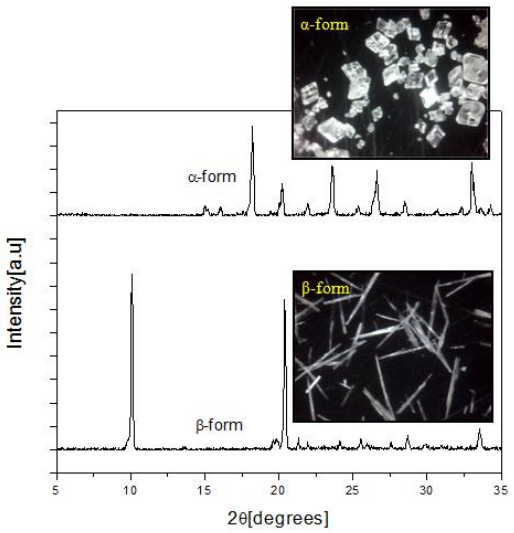

Figure 2: Structure and morphology of α-form and β-form

3.2. Heterogeneous nucleation of β-form during the phase transformation

In cooling crystallization, the α-form was initially crystallized (Fig. 3(a)) and then the β-form was generated after 5h of crystallization time (Fig.3(b)) through the solvent-mediated phase transformation of unstable α-form to stable β-form [10]. The phase transformation of α-form into β-form was completed after 40 h of crystallization time, as shown in Fig.3(c). According to our previous result [10], the nucleation of β-form was considered as an important factor impacting on the phase transformation time, where the phase transformation time would be significantly reduced if the nucleation rate of β-form was promoted. Thus, the mechanism of β-form nucleation during the phase transformation should be clearly understood.

In the current work, the mechanism of β-form nucleation was deeply studied. As shown in Fig.3(b), the β-form crystal obviously appeared on the surface of α-form crystal, meaning that the nucleation of β-form was heterogeneous nucleation which was epitaxial growth on the surface of α-form crystal. As such, the α-form crystals were known as the substrate which provided the nucleation sites for the β-form crystal. It was well known that the substrate provided a lower free energy barrier to nucleation through the favourable interactions between the substrate and solute molecules aggregate, implying that the nucleation of β-form happened on the α-form substrate was more facilitate than that occurred in the bulk solution. Moreover, since the epitaxial ordering of the β-form molecules on the α-form substrate directly depended on the functional groups of molecules on the α-form substrate surface and the lattice matching between the α-form and β-form crystal, the distinguished functional group of molecules on each α-form substrate surface and the degree of lattice matching between two polymorphs played a key role to generate the β-form nuclei.

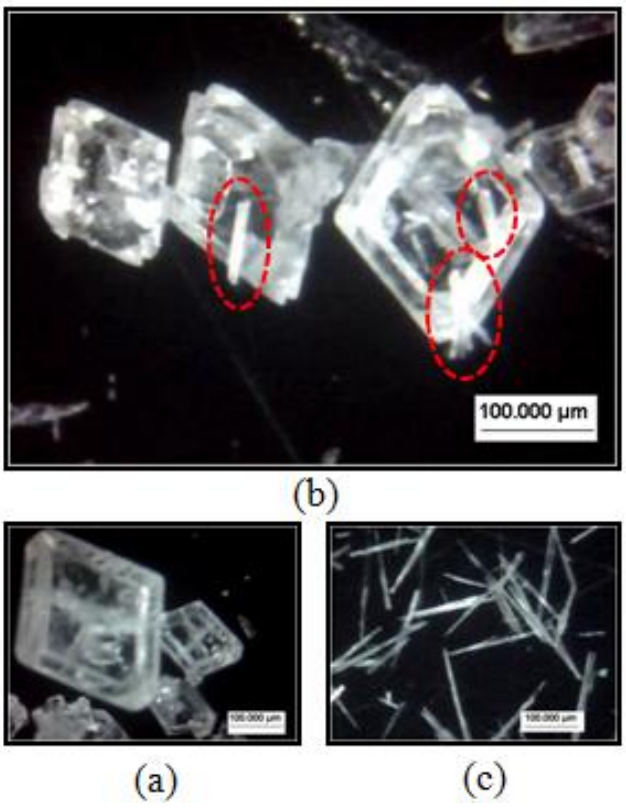

Figure 3: Heterogeneous nucleation of β-form and phase transformation of α-form to β-form

In order to understand the mechanism of β-form nucleation on the α-form crystal surface, the surfaces of α-form crystal including (001), (011) and (111) (*hkl* Miller planes) were clarified, as shown in Fig. 4. According to Fig.3(b), it was confirmed that the β-form crystal was epitaxial growth on the (001) and (011) surfaces of α-form crystal, while it had no any β-form crystals observed on the (111) surface of α-form crystal even though the experiment was repeated more than 100 times. This result implied that there was a selective nucleation of β-form on a specific surface of α-form crystal, where the β-form nuclei was favorable crystallized on the (001) and (011) surfaces rather than the (111) surface of α-form crystal.

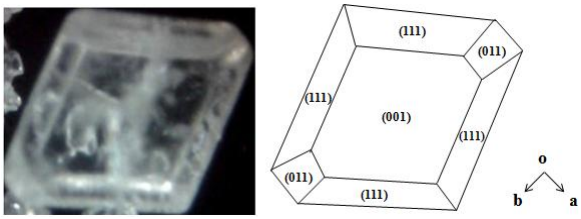

Figure 4: Schematic surfaces of α-form crystal

For deep understanding, the functional group of molecules on various surface of α-form crystal was estimated via the molecular simulation. Here, the functional groups of molecules including COO^-, OH^- and NH_3^+ oriented on each surface were simulated via the Diamon software, as depicted in Fig.5. The molecular simulation result indicated that there was significant difference with respect to the arrangement of functional groups on these surfaces. That is the functional groups C=O and O-H was observed on the (001) surface (Fig.5(a)), while it was the COO^- on the (011) surface (Fig.5(b)). Meanwhile, the functional group NH_3^+ was detected on the (111) surface of α-form crystal (Fig.5(c)). As such, the different functional groups of various surfaces of α-form crystal were a reason why there was a distinguished nucleation site for the β-form nuclei. This hypothesis was further investigated via the degree of lattice matching (E) between the β-form molecules aggregate and surface of α-form crystal as using the Grace software [7]. Here, the Grace software (global real-space analysis of crystal epitaxy) was used to calculate the degree of lattice match between two contacting α-form crystal surface and overlayer β-form lattice planes with varied θ azimuth angle, where the θ azimuth angle was defined the relative orientation between the surface lattice of α-form crystal and β-form molecules aggregate.

As shown in Fig.6, the largest value of E on various surfaces of α-form crystal including (001), (011) and (111) were 1.37, 0.68 and 0.69,

respectively, meaning that the (001) surface had a highest probability for the epitaxial growing of β-form nuclei. In case of the (011) and (111) surfaces, although the largest value of (E) on these surfaces was very competitive (0.68 and 0.69), the density of value E as $E \geq 0.56$ on the (011) surface was higher than that of (111) surface in a wide range of θ azimuth angle, where the (111) surface had only one predominant peak at $\theta = 7^0$. That means the optimum epitaxial configuration of various surface of α-form crystal for the β-form nuclei was ordered as (001) > (011) > (111). This result was consistent with the experimental result as described in Fig.3(b), where the β-form nuclei was preferably performed on the (001) and (011) surfaces instead of the (111) surface of α-form crystal.

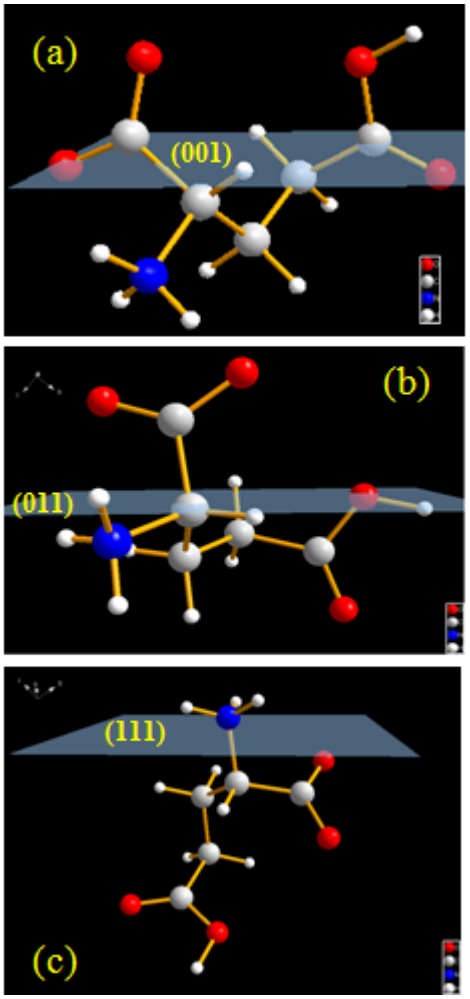

Figure 5: Functional group of various surfaces of α-form crystal: (a)-(001), (b)-(011) and (c)-(111)

The present study had a controversial result to the previous study [11], where the (011) and (111) surfaces was predominant for the β-form nuclei compared to the (001) surface. However, it should be mentioned that the crystallization in current work was carried out under stirring condition as similarity to the industrial production, while the previous study operated the crystallization under stagnant condition [11], so the mass/heat transfer and mobility of solute molecules in solution of this case were definitely different.

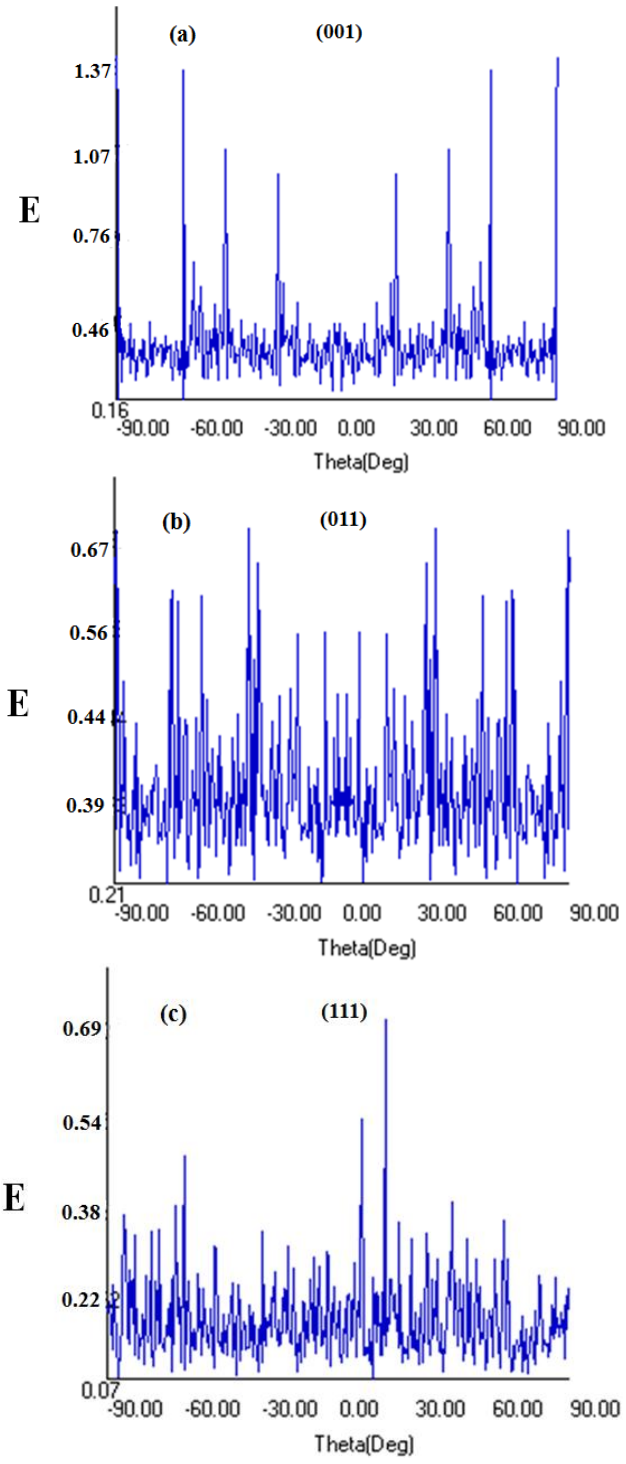

Figure 6: Degree of lattice matching between various α-form substrate surface and β-form molecules aggregate

4. CONCLUSION

The present study explored a new mechanism of β-form nucleation occurring on the substrate α-form crystal during the phase transformation of L-glutamic acid in the stirring cooling crystallization. The experimental result showed that the β-form nuclei was selectively performed on the (001) and (011) surfaces rather than the (111) surface of α-form crystal. This result matched with the molecules simulation result, where the effect of different surfaces of α-form crystal on the selective nucleation of β-form crystal was original from the distinguished functional group of molecules on various surface of α-form and degree of lattice matching (E) between the α-form substrate surface and β-form molecules aggregate. Here, the degree of lattice matching between the α-form substrate surface and β-form molecules aggregate was ordered as (001) > (011) > (111), meaning that the β-form nucleation on the (001) and (011) surfaces was more facilitated than that on the (111) surface of α-form crystal.

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